IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 1853
Bennett *et al.*	Art Unit: 1648
Appl. No. 10/622,088	Examiner: Horning, Michelle S.
Filed: July 18, 2003	Atty. Docket: IVGN 332
For: **Viral Vectors Containing Recombination Sites**	

Declaration Under 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Peter J. Welch, a resident of 4689 E. Talmadge Drive, San Diego, California declare as follows:

1. I am an inventor on the present application, together with Robert Bennett, Kenneth E. Franke, Kenneth Frimpong, Steven Harwood, and Knut Madden.
2. The invention claimed in the above identified Patent Application was conceived and reduced to practice prior to October 31, 2001, the publication date of the cited reference, Loftus *et al.*
3. Attached as Exhibit A are notebook records relating to the conception and reduction to practice of the invention. Notebook 4, pages 37-39, dated August 16 and August 17, 2001, describe the construction of the pLenti6/V5-DEST vector shown in Figure 36A of the above referenced application. The vector was constructed by ligation of an Eco RV Dest cassette into pRRL6V53stops followed by transformation into DB 3.1 cells. A similar reaction is described in the Notebook page dated October 3, 2001.
4. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of

the United States Code, and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Peter J. Welch

July 23, 2008
Date

ligation of Eco RV Dest cassette A into pRRL6V53 stops and pRRL6V53 stops ppt digested with Eco RV and CIP treated.

	1	2	3	4
10x ligation buffer	1	1.1	1.1	1.1
pRRL6V53 stops=RV	3	3	3	3
Dest cassette A	0	3	4	5
dH_2O	5	3	2	1
ligase	1	1	1	1
	10µl	~11µl	~11µl	~11µl

	5	6	7	8
10X ligation buffer	1	1.1	1.1	1.1
pRRL6V53 stops=RV (ppt)	3	3	3	3
Dest Cassette A	0	3	4	5
dH_2O	5	3	2	1
Ligase	1	1	1	1
	10µl	~11µl	~11µl	~11µl

These ligation reactions were incubated @ RT for 1 hr. 3µl was used to transform DB3.1 cells using the std. protocol

30 min on ice, heat-shocked @ 42 for 45 sec. on ice for 2'
Added 0.9 ml SOC
Incubate @ 37°C for 1h prior to plated on LB cm plates. Used pcDNA 4/HIS/lacZ as control. 200µl of each transformation was plated.

After seeing that the NruI digest of pcDNA3.1 V5HisA looked complete, I formulated a larger digest in order to generate lentivirus stocks with SV40-Neo.

10X NruI buffer@	7.0
pcDNA3.1 V5 His B	5.0
dH_2O	53.0
NruI	5.0
	70µl

this reaction was incubated @ 37°C for overnight.

Continued on Page

Read and Understood By

Signed Date Signed Date

Cadley D Howard 8/16/07 8/17/07

8/17/01 - Results of Dest vector transformations/constructions.

Transformations	# colonies
1 DB3.1 only	~~0~~
2 pcDNA4/HIS/DEST	TNTC
3 Lig 1	~~0~~
4 Lig 2	412
5 Lig 3	514 → Ber will grow up 20 colonies
6 Lig 4	1122
7 Lig 5	~~0~~
8 Lig 6	680
9 Lig 7	726 → Ber will grow up 20 colonies
10 Lig 8	765

Ber was kind enough to carry some aspects of the lentiviral project forward while I am gone on vacation.

I gave her some DNAs and some general guidelines as described on the next page.

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Read and Understood By

Bradley D Howard Signed 8/17/01 Date

Signed Date 8/17/01

Hi Ben,

Here goes!

Grow up up #6 and #15 from the up DNAs/cultures that you analyzed for me.

These plasmids should be called pRRL6FPV53.stops.ppt #6 and #15. Make Endofree preps using the Qiagen dart and protocol. Grow up 2x 100ml cultures each.

As for the pRRL6FPV53.stops, the DNAs are name pRRL6GFPV53 stops 8-2 and pRRL6GFPV53 stops 8-7. Use 8-2 since the core 1 DNA is higher. You will need to transform HB101 only, no other strain will work. Grow up ~~[redacted]~~ colonies from what results from the transformation. Label them separately. These DNAs need to be grown up using the Endofree kit (Qiagen). Remember to grow up 2 x 100 ml cultures (TB + amp 50 mg/ml).

DEST Vectors

Take ~20 colonies from plate 3, ~~[redacted]~~ These would correspond to the DEST version pRRL6V53.stops.DEST. Take 20 colonies from plate 7. These colonies would correspond to the DEST version pRRLV53.stops.ppt.DEST.

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Bradley D Howard — Signed — 8/17/01 — Date

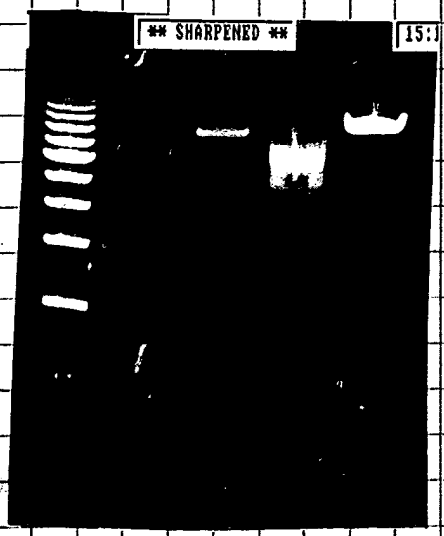
[redacted] — Signed — 8/17/07 — Date

10/1/01 SHERYLL'S HAVING SOME TROUBLE GETTING REST CASSETTE CLONES
SO I'LL TRY IN PARALLEL:

pRRL46/VS & pRRL46/VS/ppt 5µg DNA from BEV

	pRRL46/VS		pRRL46/VS/ppt	
	10µl		40µl	
H_2O	33µl		3µl	
NEB #3	5µl	→ 1.5µl	5µl	→ 1.5µl
EcoRV	2µl		2µl	
	50 (100ng/µl)		50	

37°C x 2hrs → 1.5µl



CLEAN UP / ELUTE SOLTE
USE µl/LIGATION WITH REST CASSETTE B

	VS−	VS+	ppt−	ppt+
pRRL46/VS	2.0	2.0		
pRRL46/VS/ppt	—	—	1.0	1.0
H_2O	~~4.0~~ 6	4.0	~~5.0~~ 7	5.0
10X L46 BUF	1.0	1.0	1.0	1.0
LIGASE	1.0	1.0	1.0	1.0
REST CASS. B 10ng/µl	~~2.0~~ —	2.0	~~2.0~~ —	2.0
	10			

16°C O/N

10/2/01 Thaw OB3.1 100µl AND PLATE: 100µl EACH → LB-amp
100µl " → LB-amp + Cm
REST " → LB-amp + Cm
For L46

10/3/01 EXCELLENT! VS−, ppt− = NO COLONIES
VS+, ppt+ = LOTS OF COLONIES
] ON Amp + Cm PLATES
YIPPEE!
SHERYLL WILL TAKE IT FROM HERE...

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Read and Understood By

Signed 10/3/01 Date Signed 11/7/01 Date